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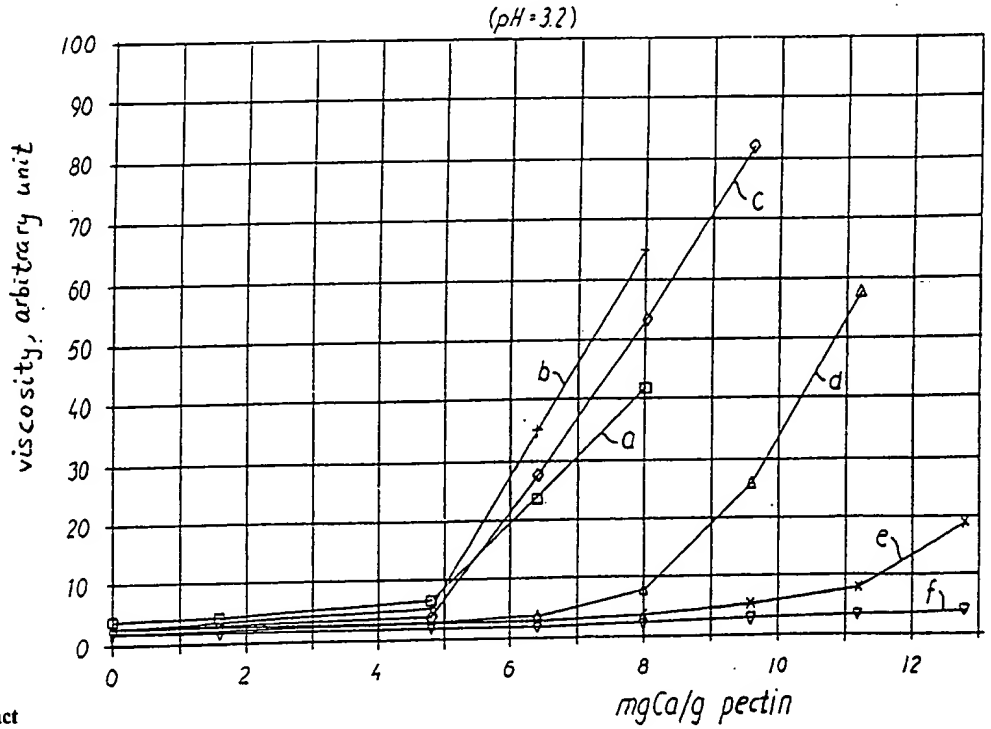

(57) Abstract

A process of improving the gelling properties of high-esterified pectin by reacting the pectin with polygalacturonase at a pH-value of 1-7 and at a temperature of 5-65°C in which the reaction is terminated before the average molecular weight of the pectin has been reduced to a value of 50 % of the average molecular weight of the starting material.

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Process of improving the gelling properties of high-esterified pectin

This invention relates to a process of improving the gelling properties of high-esterified pectin.

Conventional pectin, such as pectin produced of citrus fruits or apples, essentially consists of polymeric chains formed by polymerisation of galacturonic acid moieties the carbon atom in the 4-position in one moiety being bound to the carbon atom in the 1-position in the neighbouring moiety.

In some of the galacturonic acid moieties the carbon atoms in 6-position are esterified with methanol and if over 50% of said carbon atoms are esterified with methanol the pectin is designated high-esterified. The pectin is called low-esterified if less than 50% of the carbon atoms are esterified with methanol.

Pectin normally forms a gel if either sugar is added to obtain a concentration of more than about 60 weight percent and the pH-value is adjusted to less than about 3.1 or if a calcium solution or calcium containing fruit is added, the calcium ions being capable of functioning as a bridge between the pectin chains.

In practice, the first method is primarily used for gelling high-esterified pectins while the second method primarily is used for gelling low-esterified pectins.

To obtain a gel composition as strong and reproducible as possible it is important that the pectin is fully utilized and uniformly distributed and dissolved in the medium in which the gelling process is effected.

However, it has turned out that an optimum gel formation is not obtained by gelling some high-esterified pectins in the presence of calcium according to the first of the two above mentioned methods.

Thus, when using hard water in the solutions or mixtures to be gelled there is a tendency for the pectins to react with calcium and

to form undissolved pectin which does not contribute to the gel formation to the same degree as dissolved pectin. Consequently, the result is a weaker gel than desired.

Likewise, pectin may react with calcium when a pectin solution and fruit juices containing calcium ions are mixed prior to the initiation of the gelling process thereby increasing the viscosity of the mixture. Consequently, it is difficult to obtain a uniform distribution of pectin, and the consequence is that the subsequent gelling process does not result in a gel having an optimum strength.

An undesired gel formation of the pectin, while said pectin is being handled e.g. by stirring, pouring etc., results in that the gel structures formed are broken without the possibility of reconstructing them. Consequently, in some cases a gel cannot be obtained having the optimum strength.

As mentioned above pectin essentially consists of chains of galacturonic acid moeities and methyl-esterified galacturonic acid moeities. The sequence in which esterified and non-esterified moieties are present in the chains may vary. Segments may occur in which there is a local predominance of non-esterified moieties or in which esterified moieties may optionally be absent. It is assumed that the presence of such segments of a certain size in high-esterified pectins accounts for the calcium sensitivity of these pectins.

For most purposes pectins having a high degree of polymerisation are preferred because a better gel quality is obtained with high molecular pectins than with pectins having a lower degree of polymerisation.

Pectin produced by extraction of apple pulp has a relatively low calcium sensitivity. However, apple pulp which i.a. is formed by production of apple juice and cider is present in relatively small amounts, and the pectin content of apple pulp is considerably lower than in the peel and residues obtained after pressing of citrus fruits in which pectin is also present and which are becoming increasingly common to use for the production of pectin by

extraction. High-esterified pectin produced from citrus fruits is, however, fairly calcium sensitive.

The object of the invention is to produce high-esterified pectin having a low calcium sensitivity and a relatively high degree of polymerization from raw materials which are readily available such as peels and press residues of citrus fruits.

This object is obtained by the process according to the invention which process is characterized in that the high-esterified pectin is reacted with polygalacturonase at a pH-value of 1-7 and at a temperature of 5-65°C, and that the reaction is stopped before the average molecular weight is reduced to a value of less than 50% of the average molecular weight of the starting material.

It is well known that an enzyme preparation containing a relatively large amount of polygalacturonase is capable of splitting low-esterified pectin and undissolved, high-molecular protopectin and thereby is suitable for extracting fruit juice from juicy fruits. It is also known that this enzyme preparation only to a limited degree splits high-esterified, soluble pectin.

The invention is based on the discovery that polygalacturonase even though it is capable of splitting high-esterified pectin to a low degree as mentioned above does have an effect on said pectin, and that the result is a considerable reduction in the calcium sensitivity of the pectin without a corresponding alteration of the degree of polymerization.

It is assumed that the reason for the change in the calcium sensitivity of the high-esterified pectin is that the enzyme can easily destroy the molecular regions which in particular account for the calcium sensitivity. Surprisingly, a depolymerization of these regions does not result in a drastic reduction in the molecular weight of the pectin. This unexpected phenomenon may be accounted for if it is assumed that the regions in question are situated mainly at the ends of the molecular chains. Another possible explanation could be that a relatively small portion of the pectin molecules, which differ considerably, is responsible for the calcium

sensitivity and that particularly these molecules are decomposed by the enzyme treatment.

As mentioned above calcium ions are present in varying amounts in the fruit products which are used for manufacturing gels and jams by using pectin as a gelling agent.

The manufacturers of such gelled products do normally not know the amount of calcium ions in a given fruit material and the use of the known high-esterified pectins may therefore give rise to serious production problems.

Such production problems are by and large eliminated when using pectin produced by the method according to the above invention even when using starting materials having a relatively high calcium ion content.

The solidity of the gels can be measured by various methods which can be divided into two fundamentally different main groups:

1° destructive methods i.e. methods measuring the minimum force necessary to break the gel;

2° non-destructive methods which measure the deformation of the gel during the impact of a force which does not break the gel (i.e. the gel resumes its original shape once the effect of the force ceases).

In the destructive test the gel strength can be determined by the break stress method described in further detail in example 1.

The USA-SAG method described in the Final Report of the IFT Committee, Pectin Standarization, Food Technology, 1959, 13, 496, discloses a non-destructive method for determining the gel strength.

Gels presenting a relatively close relationship between gel strength by destructive measurement and gel strength by non-destructive measurement have an elastic texture whereas gels showing the opposite trend have a crisp texture. An elastic texture is desirable in connection with clear gels of a type particularly known in

Denmark as currant gel and rowanberry gel; in the US a corresponding grape gel is widely used.

The pectin produced according to the method of the invention offers more elastic gels when employing calcium containing systems than normally obtained by a similar pectin which has not been treated with polygalacturonase.

The method according to the invention preferably uses high-esterified pectin extracted from citrus fruits. Examples of suitable citrus fruits are lemons, oranges, lime fruits and grape fruits.

The used pectin preferably has a degree of esterification of 50-85% and the pectin is preferably used in a concentration of 0.5-8%.

Examples of particularly suitable technical preparations of polygalacturonase are Rohament® P (Röhm GmbH, BRD) and Pectinex MC (NOVO Schweitzerische Ferment AG). The enzymes are preferably used in a concentration of from 6 to 6250 PGU per gram of pectin.

As mentioned above the reaction takes place at a temperature of between 5 and 65°C. In the upper part of said temperature interval a certain destruction of the enzyme takes place concurrently with the interaction of the enzyme on the pectin thereby increasing the consumption of enzymes. On the other hand, it becomes easier to control the reaction since the reaction ceases shortly after the enzyme has been introduced. Consequently, the risk of a destruction more comprehensive than intended is reduced. A particularly preferred temperature interval is 40 to 55°C.

As mentioned above the pH-value must be between 1 and 7 and it is particularly preferable to use pH-values which are not too close to pH = 4, because the latter pH-value is the one in which the polymerization is highest. Even though the enzyme consumption optionally is higher at pH-values ranging at the ends of the above mentioned interval it may be desirable to carry out the reaction at such pH-values, e.g. at a pH-value of 2.3-3.2, in order to avoid an undesired polymerization, because the enzyme consumption normally is

relatively low.

The reaction time normally ranges from 5 minutes to 48 hours.

The reaction course is partly controlled by measuring the relative reduction in molecular weight, the reaction being terminated as mentioned above before the molecular weight of the pectin has been reduced to a value of 50% of the average molecular weight of the starting material. It is preferred to stop the reaction before the molecular weight has been reuced to 80%.

The reaction may be terminated by quickly heating the reaction mixture to about 70°C thereby causing an irreversible destruction of the enzyme. The process may also be stopped by precipitation of the pectin in a suitable solvent. However, it should be noted that the enzyme is not or only partly destroyed by this treatment. If it is desired to reduce the reaction time prior to an actual termination of the reaction this may be effected by altering the pH-value of the reaction mixture away from the optimum value for enzyme efficiency.

Normally, it will be most expedient to terminate the reaction by heat treatment as described above thereby preventing any residual active enzyme from causing a further destruction of the pectin and thus a further change of its properties when it is dissolved in water later on.

According to the invention the reaction of pectin with polygalacturonase is not terminated until

$$\frac{U \cdot T}{P} > 50 \text{ PGU} \cdot \text{min/g}$$

wherein U is the enzyme activity measured in PGU, T is the reaction time in minutes, and P is the amount of pectin used in g.

PGU (polygalacturonase activity) can be determined in the following way:

Principle

Solutions of a specified pectin are reacted with a series of solutions having different contents of the preparation with unknown polygalacturonase activity (PGU) under well defined conditions.

The molecular weight of the pectins thus treated is determined by measuring the relative viscosity of diluted solutions of pectin in a hexametaphosphate buffer.

By interpolation of the results obtained the amount of enzymes causing a 20% reduction in the initial molecular weight is determined. This amount of enzymes defines the activity of the preparation.

Method

A pectin test sample system containing 1.05% (weight/volume) of pectin and 20 mM of $CaCl_2$ and having a pH-value of 3.7 is prepared.

Pectin is used having a DE-value of from 70 to 72%, an AGA-value (anhydrogalacturonic acid content) of from 70 to 72%, and a molecular weight of from 125.000 to 135.000.

A series of enzyme solutions are prepared having various contents of enzyme preparation in a 20 mM $CaCl_2$-solution.

Each sample including the 0-sample in which a 20 mM $CaCl_2$-solution is used instead of the enzyme solution is subjected to the following procedure:

50 ml of pectin sample system is reacted with 2.5 ml of enzyme solution during stirring for 20 minutes at a temperature of 25°C.

10 ml of the sample system is mixed with 90 ml of hexametaphosphate buffer and is thoroughly stirred for 2 minutes after which the mixture is passed through a glas filter. The viscosity of the mixture formed is determined in a Ostwald-viscosimeter at a temperature of 25°C.

The apparent molecular weight is defined on the basis of the systems of formula set forth below.

Interpolation determines the enzyme concentration, C, which reduces the apparent molecular weight by 20% in relation to the molecular weight of the O-sample.

The specific activity of the enzyme preparations in PGU/mg is defined by dividing 200.000 with the specific value C.

The relative viscosity is determined by the formula

$$\eta_r = \frac{t_o - \frac{K}{t_o}}{t_m - \frac{K}{t_m}}$$

wherein t_o and t_m are discharge times for the pectin solution and hexametaphosphate solution, respectively, and K is a constant depending on the used viscosimeter.

The molecular weight of the pectin is defined on the basis of the formula:

$$M = \frac{(\eta_r^{\frac{1}{P}} - 1)\ P}{H \cdot C}$$

wherein P = 6 and H = 4.7 10^{-5} mol g^{-1} and C are % by weight of pectin in the sample system.

The value obtained is rounded up to an integer multiplum of 1000.

Litteratur: Povl E. Christensen:

Methods of Grading Pectin in Relation to the Molecular Weight (Intrinsic Viscosity) of Pectin. Food Research, Vol. 19, pp. 163-171 (1954).

Christian J. B. Smit and Edwin F. Bryant: Properties of Pectin Fractions Separated on Diethylaminoethylcellulose Columns. Journal of Food Science, Vol. 32, pp. 197-199 (1967).

The invention will be described in further detail with reference to the following examples:

Example 1: Treatment of orange pectin at two pH-values

Pectin is extracted from orange-mash. The extract is filtered through kieselguhr and the greater part of the calcium present is removed by ion exchange. The resulting extract which has a pectin content of 9 g/l is heated to 40°C and divided into two fractions which are adjusted to a pH-value of 3.2 and 4.8, respectively.

Each fraction is treated in the following manner:

(1) an aliquot is sampled and the pectin is precipitated,
(2) 0.05 ml of "Rohament®P" -solution per liter of extract is added ("Rohament®p" has a specific activity of 2500 PGU/mg); the concentration of the enzyme solution is 1 g/l,
(3) it is allowed to stand for 10 minutes,
(4) another aliquot is sampled and the pectin is precipitated,
(5) 0.10 ml of enzyme solution per liter is added to the residual fraction
(6) the above cycle is repeated, each time doubling the amount of enzyme to be added.

In this manner a number of pectins are produced in each series having an exponentially increasing degree of treatments i.e. the totals of products of enzyme concentration and treatment time in the relative ratio of 0, 1, 4, 11, 26, 57 and 120.

The pectins were analyzed for mol weight, USA-SAG, stress at break and SAG of gels at a pH-value of 3.1, partly in the presence and partly in the absence of calcium. The results obtained appear from table 1 and table 2. The calcium sensitivity was also determined by determining the viscosity of pectin solutions of 1.5 weight percent with varying calcium dosages.

The results obtained appear in Fig. 1 and Fig. 2 in which the curves a, b, c, d, e, and f correspond to the number of treatments of 0, 4, 11, 26, 57, and 120.

Table 1

Enzyme treatment at pH 3.2

Degree of treatment	Degree of esterification	Mol weight kD[1)]	USA-SAG 2)	Stress at break with Ca, pH 3.1	SAG[3)] without Ca, pH 3.1	Stress at break with Ca, pH 3.1	SAG with Ca, pH 3.1
0	68,1	112	181	38,2	141	6,9	115
1	68,3	106	179	31,8	140	13,2	122
4	68,3	105	178	34,5	141	16,8	124
11	68,6	99	168	35,5	139	24,3	121
26	68,4	95	169	27,3	136	23,6	122
57	68,9	88	157	23,6	130	26,2	120
120	69,1	85	147	8,3	115	17,3	121

Table 2

Enzyme treatment at pH 4.8

Degree of treatment	Degree of esterification	Mol weight kD[1)]	USA-SAG 2)	Stress at break with Ca, pH 3.1	SAG[3)] without Ca, pH 3.1	Stress at break with Ca, pH 3.1	SAG with Ca, pH 3.1
0	67,6	111	177	30,4	139	8,0	115
1	67,5	107	174	28,8	138	11,5	118
4	67,8	105	175	34,5	141	16,8	124
11	68,6	98	162	35,5	139	24,3	121
26	68,6	95	155	27,3	136	20,7	110
57	69,3	89	139	20,7	126	21,2	116
120	70,5	87	125	6,8	LOW	15,2	116

1) The mol weight was defined after measuring the relative viscosity of a pectin solution of 0.1% (weight/volume) under the presence of hexametaphosphate as complexing agent and on the basis of the formula: $M = 1{,}277 \cdot 10^6 (\eta_r^{1/6} - I)$ g/mol wherein η_r is the relative viscosity. The calculated values are rounded off to integer multipla of 1000.

2) The USA-SAG values were determined by a method described in the Final Report of the IFT Committee, Pectin Standardization, Food Technology, 1959, 13, 496.

3) The stress at break and SAG-values were determined by a method corresponding to the USA-SAG method but at an pH-value of 3.1 which is a pH-value typically used in connection with pectin. The stress at break measurements were made with use of an instrument known under the name Stevens LFRA Texture Analyser.

When studying the results it was found that enzyme treatment causes a reduction in the molecular weight, USA-SAG and stress at break and SAG-values at a pH-value = 3.1 in the absence of calcium ions. Contrary to this, an improvement of the stress at break and SAG values were observed at a pH-value of 3.1 in the presence of calcium ions which presents an appreciable advantage in the use of most pectins. A small increase in the degree of esterification of the pectin is observed which is due to the incomplete precipitation of the low-esterified material which has been destroyed by the enzyme.

Example 2: Relationship between loss in molecular weight and gelling properties.

This test was carried out with the purpose of elucidating the consequences of a too rough treatment of a pectinaceous extract with polygalacturonase. At intervals increasing amounts of "Rohament® P" were added to a pectin extract and aliquots were also regularly sampled for precipitation and processing of pectin. In this manner pectins having been subjected to "an adequate" and "a too rough" treatment, respectively, were isolated within the same test.

An extract from commercial production of quick-setting pectin was

used as starting material. The pectin yield from this extract was 20.0 g/l and the corresponding pectin had a degree of esterification of 71%, an anhydrogalacturonic acid content of 72.1% and a molecular weight of 109000.

600 liters of such an extract were used. The acidity was adjusted to a pH-value of 2.7 and the temperature was adjusted to 40°C.

Initially, 50 ml of a solution of 1 g "Rohament® P" per liter of water was added to the extract. After 5 minutes 10 liters of extract was sampled and immediately poured into isopropanol in order to terminate the reaction and the pectin was precipitated. Exactly 5 minutes after the first enzymes had been introduced 100 ml of enzyme solution was added to the extract. The test was continued in this manner. After each 5 minutes the double amount of enzymes was added and immediately before each addition of enzyme a fraction of the extract was sampled for precipitation and processing of pectin.

Samples 1, 3, 5, 7 and 9 were chosen from the pectin thus produced for further analysis. The results (sample 0 = untreated juice):

Pectin sample	0	1	3	5	7	9
Mol weight	109000	100000	92000	86000	87000	42000
USA-SAG	221	227	223	208	157	<140
SAG-3.1-without Ca	134	137	142	140	<100	-
SAG-3.1-with Ca	128	133	128	132	<100	-
Stress at break-3.1-without Ca	69	76	74	65	26	-

Stress at break-3.1-with Ca	78	93	73	74	22	-

The calcium sensitivity of the pectins obtained are shown in Fig. 3. Like in the other tests it appears that the treatment resulted in a noticeable decline in the tendency of the pectin solutions to become viscous in the presence of calcium.

As it appears from the above table the stress at break was improved at a pH-value of 3.1 in the presence of calcium in the mildest treatment tested. In the more rough treatment, sample 3, the molecular weight loss is so high that the stress at break in the presence of calcium is lower than in the pectin of non-treated extract. Pectin of sample 9 was subjected to such a powerful treatment that USA-SAG is lower than 140 degrees. If the pectin is to be used for gelling purposes the reduction in gelling properties is considered to be too high to justify the advantage obtained by the reduced calcium sensitivity compared to the non-treated pectin.

Example 3: Influence of pH and temperature

This test was carried out with the purpose of studying which pH-values and temperatures to use in connection with the enzyme treatment.

1 weight percent solution of commercial pectin having a degree of esterification of 71.7 was treated under the following conditions:

1A: pH = 2.5, 20°C	1B: pH = 2.5, 55°C
2A: pH = 4.0, 20°C	2B: pH = 4.0, 55°C.

The pectin solutions are subjected to treatment until a considerable but not complete reduction in the calcium sensitivity is considered to have taken place. Once this stage has been reached the solution is poured into isopropanol in order to terminate the reaction and the pectin is isolated. The purpose of the test is to obtain pectins with a comparable molecular weight and calcium sensitivity.

Sample	pH	Temp. °C	Mol weight kD	Relative Ca sensitivity[x)]
1A	2.5	20	122	4
1B	2.5	55	121	I
2A	4.0	20	107	3
2B	4.0	55	110	2

x) The relative Ca sensitivity is defined according to the following scale: 4 = most sensitive, 1= least sensitive.

It appears that a treatment at 55°C at both pH-values is preferable to a treatment at 20°C due to the fact that a more beneficial relationship between molecular weight and calcium sensitivity is obtained with the higher temperature. By comparing the results of the above table with Fig. 4 showing the calcium sensitivity of the samples obtained, it appears that a treatment at pH = 2.5 results in an more beneficial relationship between the reduction in mol weight and the reduction in calcium sensitivity than at pH = 4. Curve 0 on the figure relates to the same pectin as the one stated as the 0-sample in the subsequent example 4.

Example 4: "Pectinex MC" instead of "Rohament® P".

This test was carried out with the purpose of testing whether another commercial polygalacturonase-preparation, "Pectinex MC" from NOVO Schweitzerische Ferment AG, can be used instead of "Rohament® P". The test conditions are the same as in example 3, however with the addition of a 0 sample : pectin with a degree of esterification of 71.7 is dissolved and precipitated like the enzyme-treated pectins but not subjected to enzyme treatment. Results:

Sample	pH	Temp. °C	Mol weight kD	Relative Ca sensitivity[x)]
0	-	-	130	5
1A	2.5	20	128	3.5
1B	2.5	55	126	3.5
2A	4.0	20	100	1
2B	4.0	55	109	2

x) The relative calcium sensitivity is defined according to the following scale: 5 = most sensitive, 1 = least sensitive.

The calcium sensitivity of the samples is shown in detail in Fig. 5.

It appears that "Pectinex MC" can be used by and large with the same result as "Rohament® P".

Example 5: Enzyme treatment of slow-setting pectin extract

This example uses a pectin extract in which the pectin to be precipitated has a lower degree of esterification than in the above examples.

A pectin extract was produced of lemon-mash. The precipitation yield of the extract was 8.2 g/l. Test data for the pectin which was precipitated from the non-enzyme treated extract appear from the table below. Prior to the enzyme treatment the pH-value of the extract was adjusted to 2.5.

The extract was continuously mixed with a solution of "Rohament® P" in the ratio 7 parts of extract to 1 part of enzyme solution and the mixture was passed through a tube reactor consisting of a 200 meter armed plastics tube with an inner diameter of 19 mm and with a flow

velocity of 480 liter/hour. The temperature of the tube reactor was 53°C. The enzyme solution contained 2 g of enzyme per 100 liter. Immediately after passing through the tube reactor the extract was heated to 70°C to terminate the reaction and to inactive the enzyme completely.

Pectin[x)]	Non-treated	Treated
Molecular weight	126000	109000
USA-SAG	245	233
SAG-3.1-without Ca	126	123
SAG-3.1-with Ca	119	124
Stress at break-3.1-without Ca	106	76
Stress at break-3.1-with Ca	70	87

x) Degree of esterification 62.4.

It appears that the pectin which can be precipitated from the enzyme treated extract has higher SAG values and stresses at break at a pH value of 3.1 in the presence of calcium than the corresponding pectin of non-treated extract. In the absence of calcium the treated pectin has somewhat lower values than the non-treated.

Example 6: the Influence of Temperature

This example tested the influence of temperature,the same pectin extract being treated in a series of different temperatures.

The extract was sampled from a conventional manufactory production.

It was produced of lemon-mash. The precipitation yield of the non-enzyme treated extract was 9.5 g/l and the precipitated pectin had the following parameters: degree of esterification = 68.4, AGA% = 74.6, molecular weight = 90000.

Treatment with "Rohament® P" in a tube reactor as described in example 5, but with 1.5 g of enzyme per 100 liter enzyme solution, gave the following results:

Temp. °C	Mol weight kD	Relative Ca sensitivity[x)]
42	81	1.5
46	70	1.5
51	69	3
53	75	4.5
59	70	4.5

x) The relative calcium sensitivity is defined according to the following scale: 5 = most sensitive, 1 = least sensitive.

The calcium sensitivity of the samples is shown in detail in Fig. 6.

It is concluded that the treatment is possible at all the indicated temperatures, but most beneficial at the lower.

PATENT CLAIMS

1. A process for improving the gelling properties of high-esterified pectin, characterized in that high-esterified pectin is reacted with polygalacturonase at a pH-value of 1-7 and at a temperature of 5-65°C, and that the reaction is terminated before the average molecular weight of the pectin has been reduced to a value of 50% of the average molecular weight of the starting material.

2. A process according to claim 1, characterized in using high-esterified pectin extracted from citrus fruits.

3. A process according claims 1 or 2, characterized in using high-esterified pectin having a degree of esterification of 50-85%.

4. A process according to any of the proceeding claims, characterized in that the pectin is used in a concentration of 0.5-8%.

5. A process according to any of the proceeding claims, characterized in using a temperature of 40-55°C.

6. A process according to claim 5, characterized in that the enzyme is used in an amount of from 6 to 6250 PGU per g pectin.

7. A process according to any of the proceeding claims, characterized in using a pH-value of 2.3-3.2.

8. A process according to any of the proceeding claims, characterized in using a reaction time of from 5 minutes to 48 hours.

9. A process according to any of the proceeding claims, characterized in that the reaction is terminated before the average molecular weight has been reduced to 80% of the average molecular weight of the starting material.

10. A process according to claim 1, characterized in that the reaction is terminated by heating the reaction mixture to at least 70°C.

11. A process according to any of the proceeding claims, characterized in that the reaction of pectin with polygalacturonase is not terminated until

$$\frac{U \cdot T}{P} > 50 \text{ PGU min./g}$$

wherein U is enzyme activity measured in PGU, T is time measured in minutes, and P is the amount of pectin used in g.

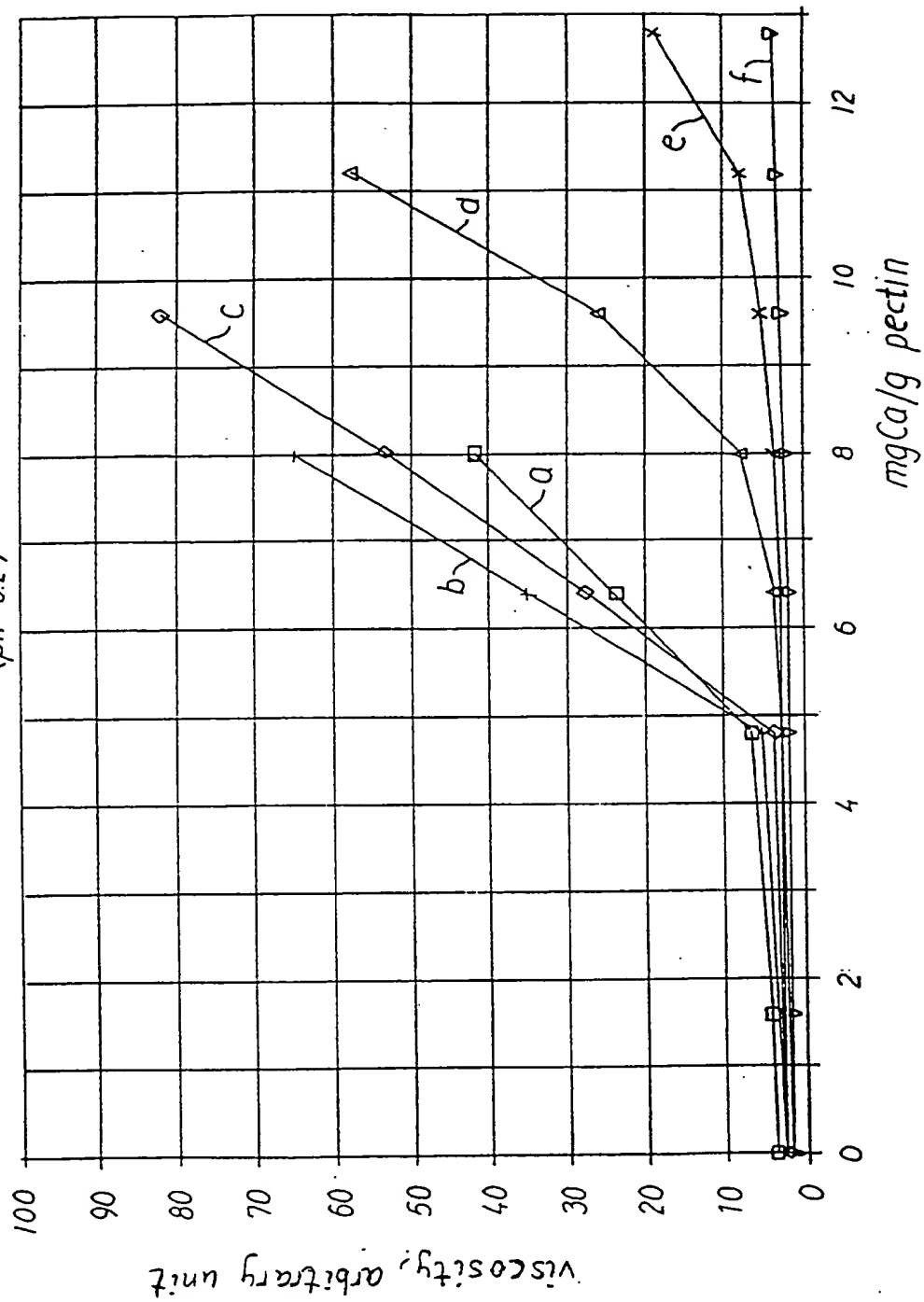
viscosity, arbitrary unit
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
mgCa/g pectin
a
b
c
d
e
f

FIG. 1
(pH = 3.2)

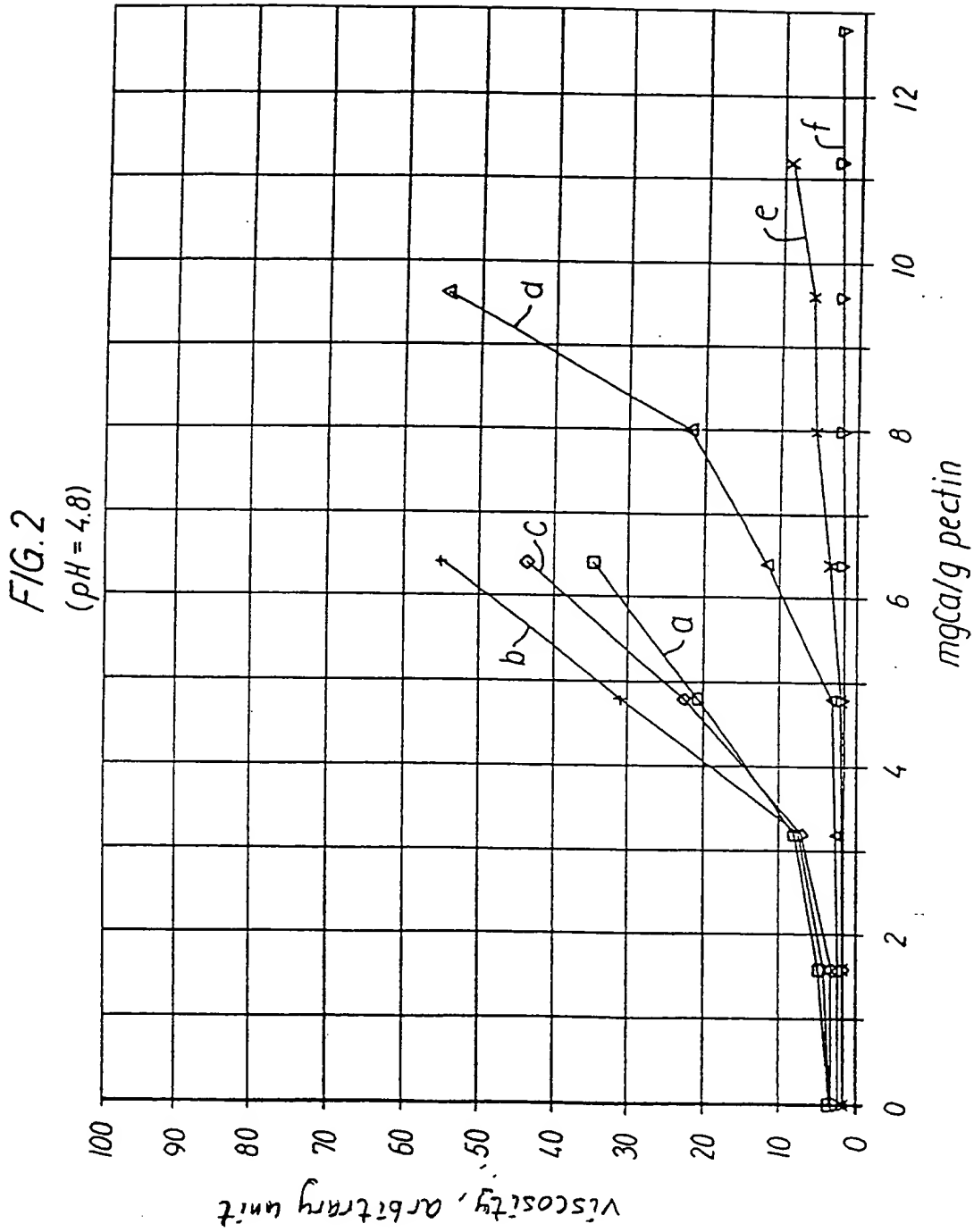
Viscosity, Brabender unit
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
mgCa/g pectin
a
b
c
d
e
f

FIG. 2
(pH = 4.8)

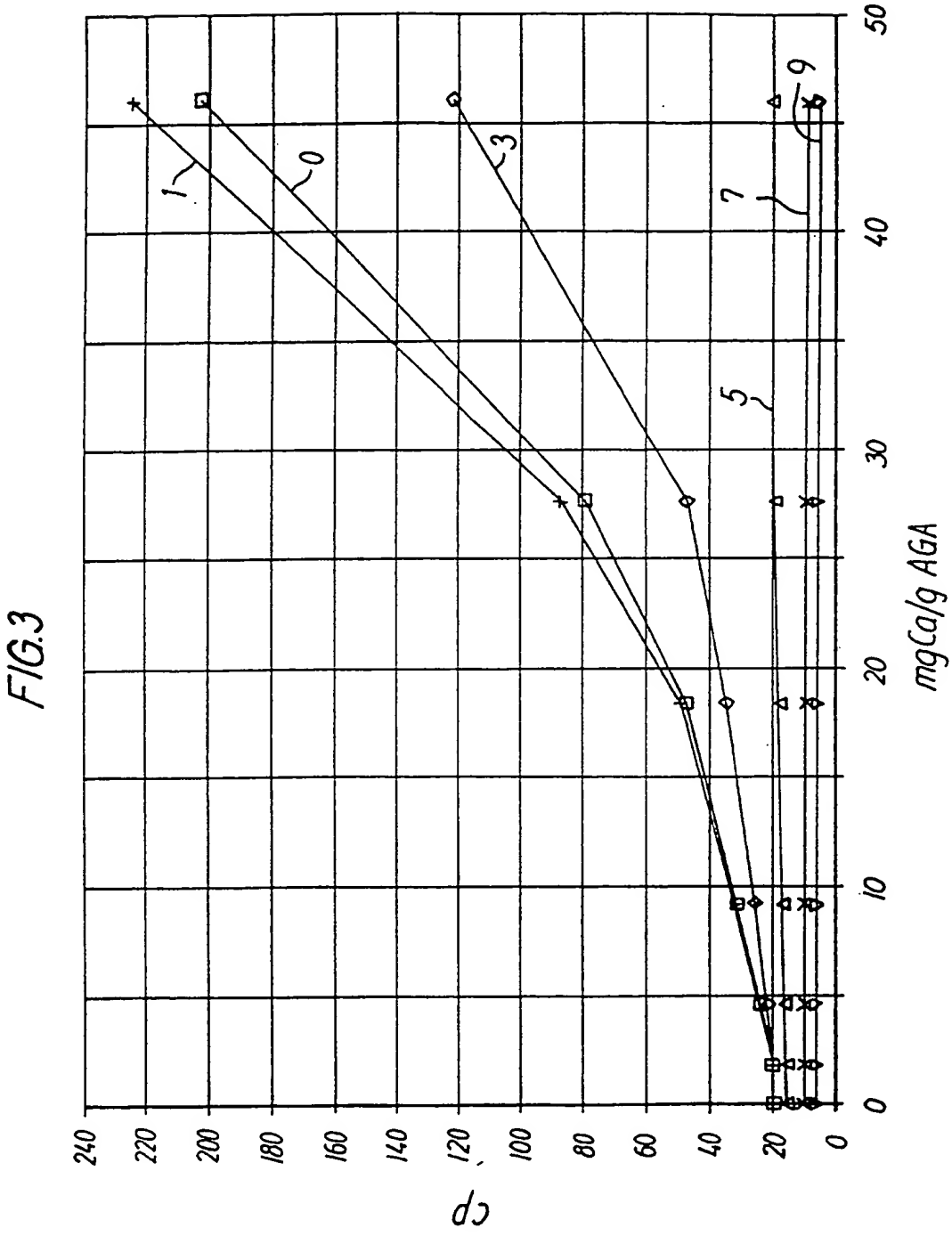
0
10
20
30
40
50
mgCa/g AGA
240
220
200
180
160
140
120
100
80
60
40
20
0
cp
1
0
3
5
7
9

FIG.3

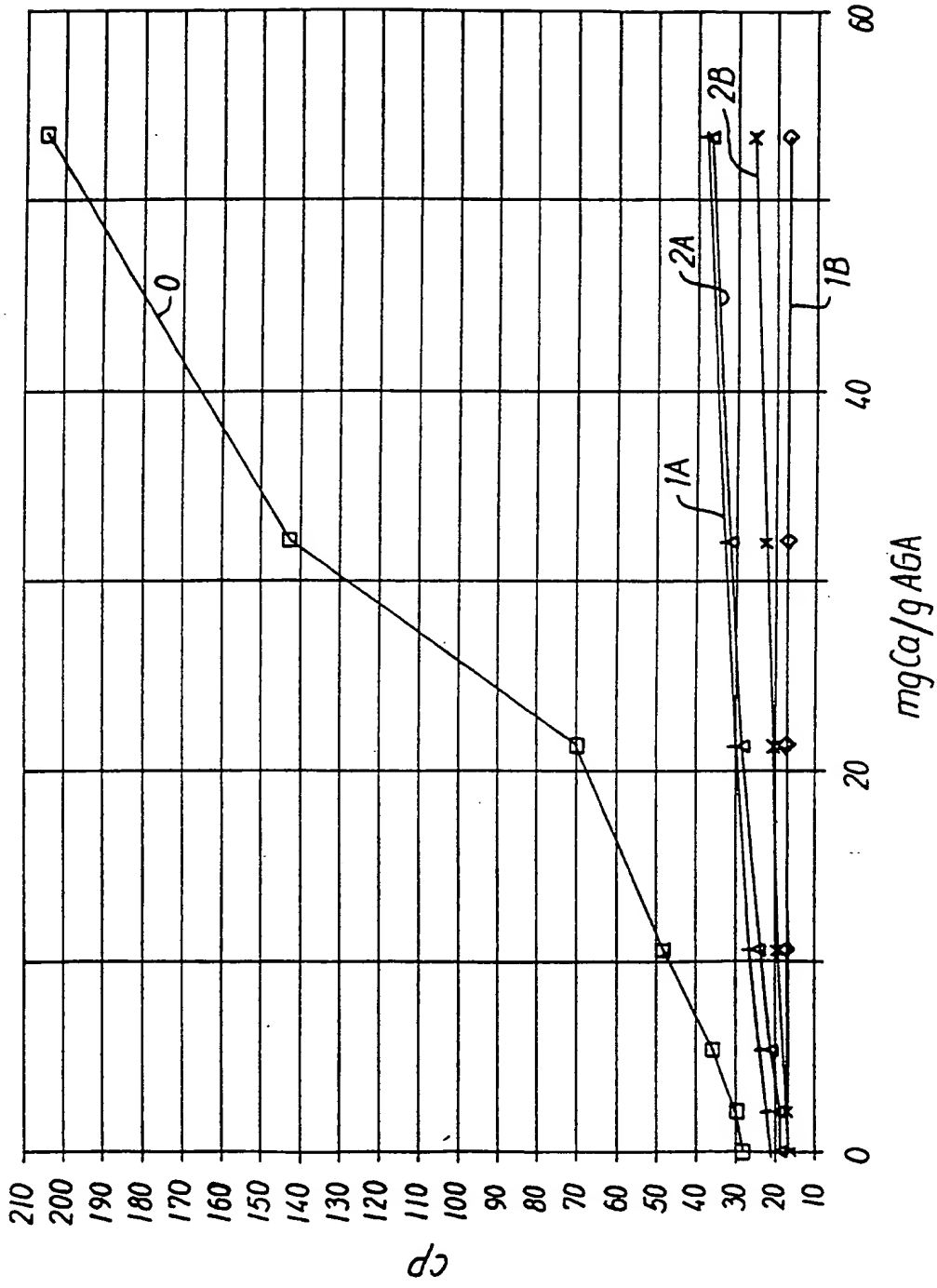
0
1A
2A
1B
2B
0
20
40
60
mgCa/g AGA
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
cp

FIG. 4

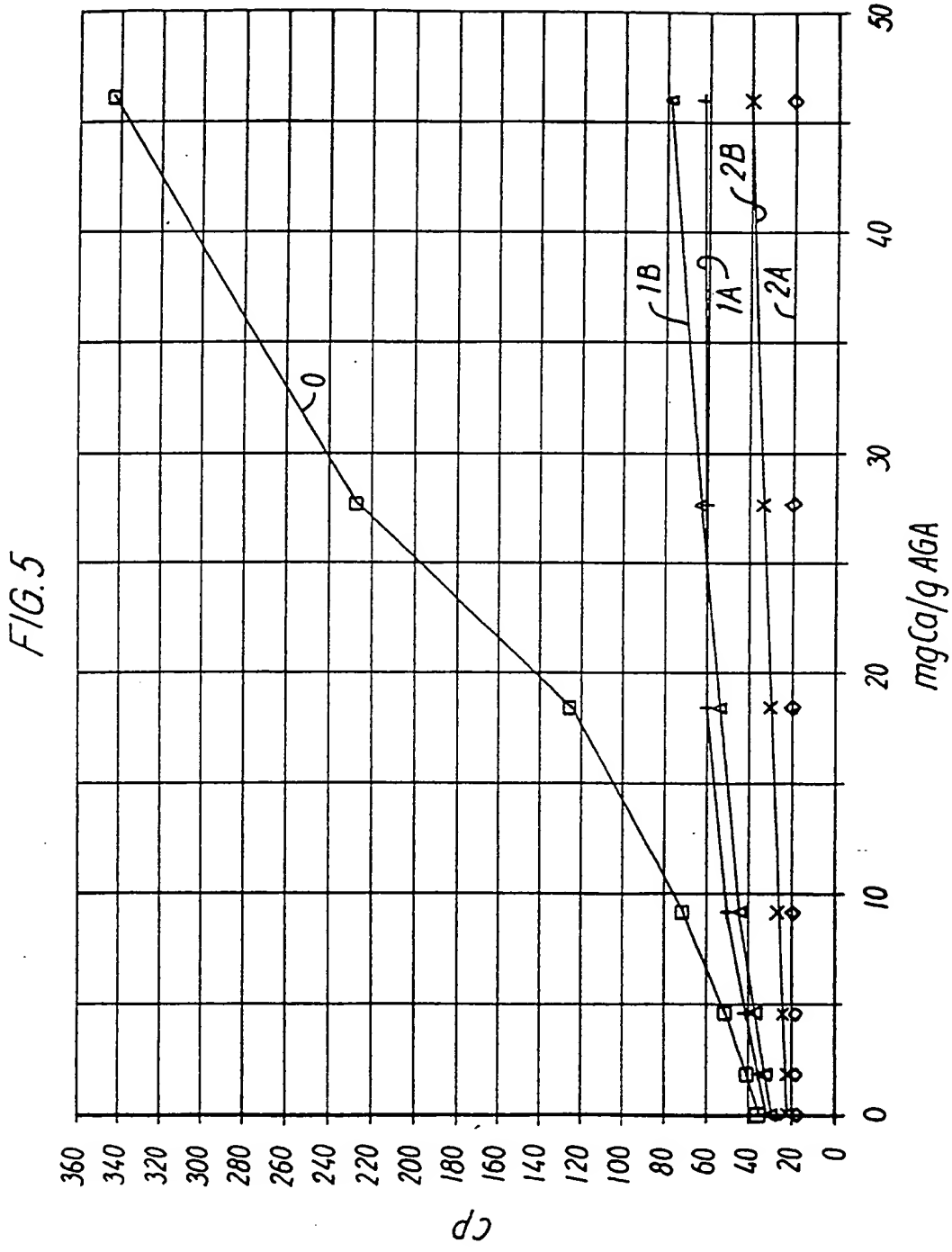
0
1B
1A
2B
2A
0
10
20
30
40
50
mgCa/g AGA
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
cp

FIG. 5

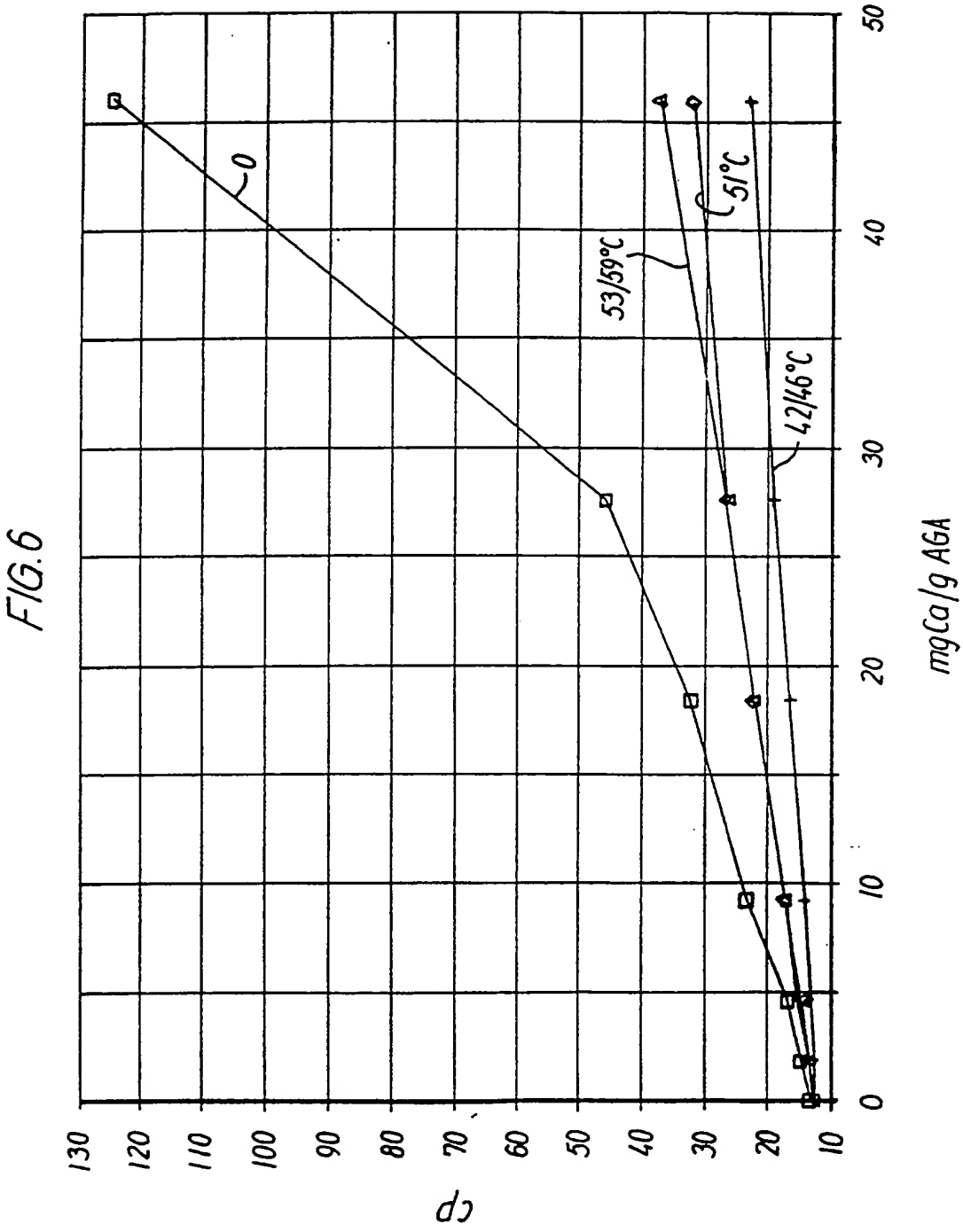
0
51°C
53/59°C
42/46°C
130
120
110
100
90
80
70
60
50
40
30
20
10
cp
0
10
20
30
40
50
mgCa/g AGA

FIG.6

INTERNATIONAL SEARCH REPORT

International Application No PCT/DK89/00159

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC 4

C 08 B 37/06, C 12 P 19/04

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	– Classification Symbols
IPC 4	C 08 B 37/06, C 12 P 19/04
US Cl	536:2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

SE, NO, DK, FI classes as above.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	DE, A1, 2 843 351 (KIKKOMAN SHOYU CO LTD) 12 April 1979 page 11, lines 5-8, page 17, last paragraph; page 18 last paragraph, the claims & FR, 2405263 JP, 54055784 US, 4200694 JP, 54055792	1-2,5,7-9
Y	Chemical Abstracts, Vol 103 (1985), abstract No 67444m, Ger.(East) DD 216,955	1-2,5,7-9
Y	Chemical Abstracts, Vol 76 (1972), abstract No 96126p, J.Gen.Appl. Microbiol. 1971, 17(5), 421-7 (Eng).	1,8
A	Chemical Abstracts, Vol 107 (1987), abstract No 171224h, Prikl.Biokhim. Mikrobiol. 1987, 23(4), 561-7 (Russ).	1-11

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
1989-09-29	1989 -10- 02
International Searching Authority: Swedish Patent Office	Signature of Authorized Officer: Agneta Österman Wallin

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Chemical Abstracts, Vol 95 (1981), abstract No 40943c, Sci.Aliments 1981, 1(1), 81-9 (Eng).	1-11
A	Chemical Abstracts, Vol 90 (1979), abstract No 181916e, J. Food Sci. 1979, 44(2), 611-14 (Eng)	1-11

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